Effect of Oxymetholone by the Mother Treatment During Pregnancy and Lactation in the Testicular Tissue in Mature Male Rats

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Abstract: Introduction: Oxymetholone is an active nutritional anabolic - androgenic steroid which is used as a performance-enhancing drug in high dose and may result in the lung cancer, mineral deposits in the lungs, irregular cycles, ovarian and liver cancer. Unfortunately, some athletes take oxymetholone as a performance-enhancing drug for its anabolic effects and muscle growth in high doses. The aim of this study is to investigate oxymetholone induced complications in the testicular tissue in immature male rats, whose mothers were treated by oxymetholone during different pregnancy and lactation periods.

Materials and Methods: In this study, we used fifty six female rats and fourteen male rats aged 110-120 days, weighing were approximately 200 ±20g. The animals were divided into groups including control, solvent 1 (pregnancy - 21 days), solvent 2 (pregnancy - lactation - 42days) solvent 3 (lactation - 21 days), experimental 1 (pregnancy 21 days) experimental 2 (pregnancy - lactation - 42 days), and experimental 3 (lactation-21 days). Solvent groups used DMSO with 99.5% concentration and experimental groups used oxymetholone with 10 mg/kg concentration by the intra peritoneum method.

Results: The rate of spermatocyte cells in the experimental group of pregnancy - lactation and spermatid cells in the groups of pregnancy and pregnancy - lactation indicated a significant decrease compared to control.

Conclusion: Oxymetholone in high doses or long-term influences on tissues and physiological activities in the reproductive system.

Keywords: Oxymetholone, Spermatogenesis, Rats.

Introduction
In the 1930s anabolic steroids were developed to treat gonads hypothyroidism in males. In this disease, the testicles do not produce sufficient testosterone (the male sexual hormone). As a result, the patient's growth and sexual acts are impaired (16). In the 1930s, scientists discovered that anabolic steroids can promote the growth of skeletal muscle in laboratory animals. After this discovery, lifters and bodybuilders and then athletes in other sports used the compounds (11,22). Androgenic-Anabolic steroids have similareffects as testosterone and dihydrotestosterone (1,7). As the name androgenic-anabolic steroid denotes, it has two different overlapping properties. Anabolic property denotes the anabolism and androgenic activity denotes that they have an effect on the development and maintenance of masculine characteristics (15). Some of the anabolic effects of these drugs are increased production of red blood cells which result in the formation of muscle cells and consequently in increased muscle mass and force (13). One of these analogues of testosterone is oxymetholone which was synthesized for the first time by Ringold et al., in 1959. It was 17α-Alkylation derivatives of testosterone from a major category of anabolic drugs. Oxymetholone is an active nutritional anabolic - androgenic steroid which is synthesized by α17 carbon...
methylation and -α5 testosterone saturated carbon.

In the 2 carbon position, it also belongs to the hydroxymethyl group. The drug is already sold by the Anadrol brand (3). It also used for acute infections, burns, trauma, surgery, recovery and underweight treatment of these diseases (13).

Athletes use it for building muscle and increasing strength, power and aggression in high doses (19). Some of the side effects of this drug include: The emergence of early baldness, aggressiveness, liver tumors, sadness, resentment, heart hypertrophy and madness (12, 21). Its use in adolescents may increase the frequency and intensity of erections as well as premature sexual maturity (6).

Some studies regarding the effect of this drug on testicular tissue structure suggest testicular atrophy and reduced number of germ cells (2). Oxymetholone consumption during pregnancy and lactation reduces the sex hormone in female newborn rats (17). The amount of LH and FSH and testosterone reduces in male newborn rats (9, 14).

Given that oxymetholone is a derivative of testosterone, so extensive research about other various tissues including the genital tract which survival depends on it appears essential. Therefore, in the case of dangerous effects during pregnancy and lactation in the new born rats, anabolic can be a good solution to prevent the indiscriminate use of the drug in different individuals. The present study aimed to investigate the induced complications by oxymetholone on the testicular tissues of mature male animals.

Materials and Methods
This is a completely random laboratory research. In line with this experimental study, all ethical principles about how to work with laboratory animals have taken into consideration. For this study, 56 female rats and 14 Wistar male rats at the age of 120-110 days and weighing approximately 20 ± 200 g from the center of breeding and keeping laboratory animals in Shiraz University of Medical Sciences were used.

The animals were kept in animal houses for two weeks before the experiment for adaptation. The animals were fed by compact palette from Shiraz company. Temperature of ambiance was 22±2°C and humidity was 50-55%. Also 12 hours of light and 12 hours of night/darkness were considered and the animals were kept in specific cages. The cages were cleaned up and disinfected once a day. The animals were divided into 7 groups of 8 including control, solvent 1 (pregnancy -21 days), solvent 2 (pregnancy - lactation-42 days) solvent 3 (lactation - 21 days), experimental 1 (pregnancy 21 days) experimental 2 (pregnancy - lactation - 42 days), and experimental 3 (lactation-21 days).

Control groups were only fed by standard laboratory water and food ad libitum. Solvent groups injected DMSO as Oxymetholonesolvent during pregnancy, lactation and pregnancy and lactation periods by the intra peritoneum method.

All injections and animal dissection were conducted within 12-10 hours of the morning. The experimental groups of pregnancy, lactation and pregnancy, and lactation were injected oxymetholone at a concentration of 10 mg kg based on the previous studies (7) during pregnancy, pregnancy and lactation and lactation periods by intraperitoneal method respectively. Newborn rats were weighted by digital scale with 0/001 accuracy (AND brand Japan). In all groups, after a month of birth, their testes were removed and after fixation, the samples were cut and stained with hematoxylin and eosin.

The results of the measurements and the cell counts were in the form of raw data on the computer. The findings were analyzed by software SPSS version 16 and one-way ANOVA and Duncan (3). The mean and standard deviation were calculated, and the value of P <0/05 was considered as statistically significant. According to the Duncan, if there is a common letter, there is no significant difference. Chart were plotted by software.

Findings
The results obtained from this study showed that the number of spermatocytes cells in
the experimental group of lactation-pregnancy indicated a significant decrease (P <0.05) compared with the control group (Figure 2). The number of spermatid cells in the experimental groups of pregnancy and pregnancy - lactation indicated significant decrease (P <0.01) compared with the control group (Figure 3).

**Conclusion**

Androgenic anabolic steroids occupy receptors and cause negative feedback to the brain and hypothalamic - pituitary - gonadal axis is inactivated and stimulating hormones in testes are not produced and secreted (19). As a result, the effects of testosterone on the testis including various levels of spermatogenesis, maintenance and tissue integration of the seminiferous tubules are disturbed and spermatogenesis is conducted on a regular basis. Other studies show that stress during pregnancy for example by injections or oral medications can inhibit the activity of the testicles and reduce the concentration of testosterone and also decreased levels of LH in the rat progeny. It is possible to reduce this stress by reducing the secretion of norepinephrine in the hypothalamus LH and testosterone in male rats (21,5).

There are reports that Leydig cells act as a target cell for factors such as vasopressin, IL (IGF) and unknown factors are drivers for Sertoli cells. Thereby reducing the number and function of Leydig cells also impairs the secretion of testosterone and other testicular functions (10, 8). In the present study the number of these cells has been reduced. Reducing the number of Leydig cells could be due to their interaction with Sertoli cells and failure to reduce the number of Sertoli cells could be due to the resistance of cells against various environmental factors. Reports suggest that spermatogenesis depends on a series of cell to cell interactions (18).

Among other things, Leydig and Sertoli cells interactions can be mentioned. Research shows that the decrease in Leydig cells and cellular factor secretion by Leydig cells leads to increased destruction of spermatocytes.

Studies by vinkoskie have suggested that the seminiferous tubules regulate secretory function of Leydig cells (20). So it must be admitted that the decrease in Leydig cell function reduced spermatogenesis progress (8).

From the findings of this study it can be concluded that oxymetholone decreased significantly the number of spermatocytes during pregnancy along with lactation and a significant reduction in the number of spermatid cells during pregnancy and pregnancy along with lactation and significant reduction in the number of Leydig cells during pregnancy along with lactation which is most likely due to induction of negative feedback in the hypothalamic-pituitary-gonadal.

**Figure 1:** The effect of oxymetholone in the number of spermatogonic cells in the control and experimental groups. Groups with a common minimum level of 5% in Duncan test show no significant difference.
Figure 2: The effect of oxymetholone in the number of spermatocyte cells in the control and experimental groups. Groups with a common minimum level of 5% in Duncan test show no significant difference.

Figure 3: The effect of oxymetholone in the number of spermatid cells in the control and experimental groups. Groups with a common minimum level of 5% in Duncan test show no significant difference.

Figure 4: The effect of oxymetholone in the number of sperm cells in the control and experimental groups. Groups with a common minimum level of 5% in Duncan test show no significant difference.
Figure 5: The effect of oxymetholone in the number of serotil cells in the control and experimental groups. Groups with a common minimum level of 5% in Duncan test show no significant difference.

Figure 6: The effect of oxymetholone in the number of Leydig cells in the control and experimental groups. Groups with a common minimum level of 5% in Duncan test show no significant difference.

Figure 7: Reduced number of spermatocyte, spermatid and Leydig in the experimental group of pregnancy-lactation compared with the control group. (Staining: H & E- Magnification: X400)

Figure 8: Reduced number of spermatid in the experimental group of pregnancy compared with the control group. (Staining: H&E- Magnification: X 400)
Investigation of seminiferous tubules in the experimental group compared with the control group 1 shows that the number of spermatid cells in the experimental group 1 showed significant decrease in the number of spermatid cells in the control group.

Investigation of seminiferous tubules in the experimental group 2 compared with the control group shows that the number of spermatocytes, spermatids and Leydig cells of the experimental group 2 showed significant decrease with the number of spermatocytes, spermatids and Leydig cells in control group.

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Investigation of seminiferous tubules in the experimental group 3 compared with the control group shows no significant difference in terms of sexular, leydig and sertolie cells.

Investigation of seminiferous tubules in the experimental group 1 compared with the control group shows no significant difference in terms of sexular, leydig and sertolie cells.

Investigation of seminiferous tubules in the experimental group 2 compared with the control group shows no significant difference in terms of sexular, leydig and sertolie cells.

Figure 12: Photomicrograph of seminiferous tubules in mature control group 1. (Staining: H&E- Magnification: X400)

Figure 13: Photomicrograph of seminiferous tubules in mature control group 2. (Staining: H&E- Magnification: X400)

Figure 14: Photomicrograph of seminiferous tubules in mature control group 3. (Staining: H&E- Magnification: X400)
Investigation of seminiferous tubules in the experimental group 3 compared with the control group shows no significant difference in terms of sexular, Leydig and Sertoli cells.

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